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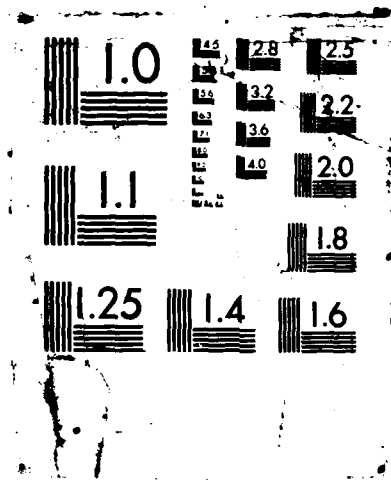
INFLUENCE OF ALTITUDE AND CAFFEINE DURING REST AND
EXERCISE ON PLASMA LEV. (U) ARMY RESEARCH INST OF
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The purpose of this study was to examine the resting and exercise response patterns of plasma Peptide F immunoreactivity (ir) to altitude exposure (4300m) and caffeine ingestion (4mg·Kg⁻¹·BW⁻¹). Nine healthy male subjects performed exercise tests to exhaustion (80 - 85%VO₂max) at sea level (50m), during an acute altitude exposure (1 hr. hypobaric chamber.4300m) and after a chronic (17 day sojourn.4300m) altitude exposure. Using a randomized, double-blind/placebo experimental design, a placebo or caffeine drink was ingested 1 hour prior to exercise. Exercise (without caffeine) significantly (p 0.05) increased plasma Peptide F ir values during exercise at chronic altitude only. Caffeine ingestion significantly increased plasma Peptide F ir concentrations during exercise and in the post-exercise period at sea level. Conversely, caffeine ingestion at altitude resulted in significant reductions in the post-exercise plasma Peptide F ir values. The results of this study demonstrate that the exercise and recovery response patterns of plasma Peptide F ir may be significantly altered by altitude exposure and caffeine ingestion. These data support further

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study examining relationships between Peptide F (and other ECPs) and epinephrine release in response to these types of physiological stresses.

**Influence of Altitude and Caffeine During
Rest and Exercise on Plasma Levels of
Proenkephalin Peptide F**

**William J. Kraemer¹, Paul B. Rock², Charles S. Fulco²,
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Kraemer, W.J., P.B. Rock, C.S. Fulco, S.E. Gordon, J.P. Bonner, C.D. Cruthirds, L.J. Marchitelli, L. Trad and A. Cymerman. Influence of altitude and caffeine during rest and exercise on plasma levels of proenkephalin Peptide F. Peptides.... The purpose of this study was to examine the resting and exercise response patterns of plasma Peptide F immunoreactivity (ir) to altitude exposure (4300m) and caffeine ingestion ($4\text{mg} \cdot \text{KgBW}^{-1}$). Nine healthy male subjects performed exercise tests to exhaustion ($80 - 85\% \dot{V}O_{2\text{max}}$) at sea level, (50m) during an acute altitude exposure (1 hr, hypobaric chamber (4300m)) and after a chronic (17 day sojourn (4300m)) altitude exposure. Using a randomized, double-blind/placebo experimental design, a placebo or caffeine drink was ingested 1 hour prior to exercise. Exercise (without caffeine) significantly ($p < 0.05$) increased plasma Peptide F ir values during exercise at chronic altitude only. Caffeine ingestion significantly increased plasma Peptide F ir concentrations during exercise and in the post-exercise period at sea level. Conversely, caffeine ingestion at altitude resulted in significant reductions in the post-exercise plasma Peptide F ir values. The results of this study demonstrate that the exercise and recovery response patterns of plasma Peptide F ir may be significantly altered by altitude exposure and caffeine ingestion. These data support further study examining relationships between Peptide F (and other ECPs) and epinephrine release in response to these types of physiological stresses. *Keep extra...*

Endogenous opioid peptides, altitude acclimatization, lactate, cardiovascular, caffeine, endurance exercise.



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Plasma Peptide F [preproenkephalin-(107-140)] levels have been shown to increase in response to endurance exercise and environmental heat stress (7-9). Although the physiological role of Peptide F remains speculative, a greater understanding of its response patterns to physiological stresses is emerging. These responses appear to be related to endurance fitness, thermal heat stress and the intensity of the exercise stress (7,9).

It has been demonstrated that enkephalin-containing polypeptides (ECP's), found in the adrenal medullary chromaffin cells, are secreted in response to the same stimuli that induce epinephrine release (6, 12, 17). Thus, it might be hypothesized that physiological stressors that are known to stimulate catecholamine release, such as exercise, high altitude and caffeine ingestion (2,3,10,13) would also produce alterations in Peptide F release.

In order to gain further insights and a greater understanding regarding plasma response patterns of Peptide F immunoreactivity (ir), this study was undertaken. The purpose of this investigation was to examine the resting, exercise and recovery response patterns of plasma Peptide F ir to: 1. caffeine ingestion and 2. acute and chronic altitude exposures.

Methods

Nine healthy male subjects volunteered as subjects for this study. Subjects were medically screened and gave written informed consent to participate in the study. All of the subjects were life-long residents at low altitude. The physical characteristics of the subjects were (mean \pm 1 SD): age (years) 20.55 ± 2.24 , height (cm) 163.0 ± 13.17 , weight (Kg) 73.54 ± 7.44 .

Each subject was familiarized with all experimental procedures prior to testing. Experimental testing was conducted in the hypobaric environmental chamber at the U. S. Army Research Institute of Environmental Medicine, Natick, Massachusetts and at the U. S. Army Pikes Peak Laboratory at the summit of Pikes Peak, Colorado.

Submaximal (two tests) and maximal (one test) exercise tests were performed at each ambient altitude [i.e. at sea level (50m), during simulated altitude exposure of 1 hour (4300m) and during the last four days of a seventeen day sojourn at altitude (4300m)]. Data from the maximal exercise test were utilized to determine and monitor the relative exercise intensity of the submaximal exercise tests. The test for maximal oxygen consumption consisted of a continuous incremental test protocol using an electronically braked cycle ergometer (Collins, Inc.). Each test started at 50 watts (60 rpm) and was increased 25 watts every two minutes until voluntary exhaustion. Oxygen uptake was measured throughout the test using a Sensormedics Horizon Metabolic Cart System (Sensormedics Corp.) which was calibrated prior to each test with standard high purity gases. Subjects breathed into a low resistance valve and cardiorespiratory responses were continuously monitored. Heart rate was monitored each minute via ECG. The heart rate and cardiorespiratory variables recorded pre-exercise, mid-exercise (10 min into exercise) and at voluntary exhaustion (last min of exercise) were used for statistical analysis.

Using the identical exercise and metabolic equipment previously described, high intensity submaximal tests (i.e. with and without caffeine) were performed at each ambient altitude exposure (i.e. sea level, acute altitude and chronic altitude). This test consisted of continuous cycle exercise (60 rpm) maintained between 80-85% of the ambient $\dot{V}O_{2\max}$ value until voluntary exhaustion. Two submaximal tests were

separated by 2 days of rest to allow for complete recovery. A randomly assigned double blind/placebo experimental design was utilized to evaluate the effects of caffeine. One hour prior to each submaximal test, subjects drank a solution containing either $4 \text{ mg} \cdot \text{KgBW}^{-1}$ of caffeine or a placebo drink (10). Additionally, prior to all submaximal testing, a 20 gauge teflon catheter was inserted into an antecubital arm vein. The catheter was kept patent by a continuous flow of isotonic saline.

Blood samples were obtained immediately prior to exercise, (after a 15 min equilibration period [9]) mid-exercise (10 min into exercise) and 5 minutes post-exercise. Blood samples (3 ml) for Peptide F analysis were obtained using cooled plastic syringes which contained sodium heparin and aprotinin ($25 \mu\text{l} \cdot \text{ml}^{-1}$ whole blood)[Sigma Chemical Co.]. The blood was gently mixed and centrifuged at $1500 \times g$, 4°C for fifteen minutes. Plasma samples were stored at -120°C until analyzed. Hemoglobin and hematocrit were determined via a 5880 mico Coulter Counter in triplicate. Blood lactate was measured in triplicate using a Lactate Analyzer-640 (Wolverine Medical Inc.). Changes in plasma volume ($\%\Delta\text{PV}$) were calculated from changes in hematocrit and hemoglobin (4).

The methods used to conduct the radioimmunoassay for Peptide F have been previously described in detail (8, 11). Briefly, Peptide F was measured by radioimmunoassay in duplicate using commercially available ^{125}I ligand and antisera (Peninsula Laboratories, Belmont, CA). This antisera showed the following cross-reactivities: 0.047%-Met-enkephalin, 0.046% - Leu-enkephalin, 0.041% - Neo-endorphin, 0.037% - Dynorphin¹⁻¹⁷ and 0.034% β -endorphin (7). The plasma immunoreactivity showed parallel displacement to Peptide F. The mean percent recovery of radioactively

labeled Peptide F with this procedure was 85%. All samples were measured in the same radioimmunoassay to avoid run to run assay variations (i.e. interassay variations). Intra-assay variations were less than 5%. Determination of plasma ir were accomplished with the use of a Beckman 5500 Gamma Counter and data reduction system.

Statistical evaluation of the data was accomplished by a 3x2 Analysis of Variance (ANOVA) with repeated measures or paired "t" tests where appropriate. Subsequent post-hoc analyses for the ANOVA were performed using a Tukey test. Significance was chosen as $p < 0.05$.

Results

The subjects in this investigation were all unacclimatized to altitude exposure prior to this study. All subjects exhibited expected responses to acute and chronic altitude hypoxia exemplified by changes in $\dot{V}O_{2\max}$, hemoglobin and hematocrit (14). Maximal oxygen consumption ($\dot{V}O_{2\max}$) was significantly reduced from sea level values ($50.37 \pm 6.29 \text{ mL} \cdot \text{Kg}^{-1} \cdot \text{min}^{-1}$) at acute altitude exposure ($37.26 \pm 3.17 \text{ mL} \cdot \text{Kg}^{-1} \cdot \text{min}^{-1}$) and after chronic altitude exposure ($38.95 \pm 3.22 \text{ mL} \cdot \text{Kg}^{-1} \cdot \text{min}^{-1}$). There were no significant differences between the acute and chronic altitude values for $\dot{V}O_{2\max}$. Significant increases in resting hemoconcentration were also observed and evidenced by significant increases in hemoglobin and hematocrit values (mean \pm 1 SD) following chronic altitude exposure: sea level ($14.6 \pm 0.7 \text{ gm/dL}$ and $43.0 \pm 1.9\%$), acute altitude ($14.7 \pm 1.1 \text{ gm/dL}$ and $43.4 \pm 3.3\%$) and chronic altitude ($17.6 \pm 1.2 \text{ gm/dL}$ and $51.0 \pm 3.6\%$). Table 1 summarizes the exercise responses of selected metabolic and cardiovascular measures to high intensity submaximal exercise at the three altitude exposures with and without caffeine.

Figure 1 shows resting, exercise (mid) and recovery response patterns of plasma Peptide F ir to caffeine and three altitude exposures. Exercise significantly increased plasma Peptide F ir above rest only at the "mid" timepoint (i.e. during exercise) with chronic altitude exposure (Pikes Peak) for the "without caffeine" trials. In the "caffeine" trials exercise significantly increased plasma Peptide F ir at the mid and post-exercise timepoints for sea level and at the mid timepoint for acute altitude.

Caffeine ingestion significantly increased plasma Peptide F ir at sea level for the mid- and post-exercise timepoints and significantly decreased values at chronic altitude (Pikes Peak) for the post-exercise timepoint.

Altitude exposure (both acute and chronic) produced a significant decrease in plasma Peptide F ir at the post-exercise timepoints during the "caffeine" trials only.

Discussion

The comparative sea level and altitude cardiorespiratory and metabolic responses in the present study agree with previous work (14,15). The results of this study indicated that altitude (4300m) exposure alone had no observable effects on resting plasma Peptide F ir values. Since ECPs have been shown to be responsive to the same stimuli which results in epinephrine release, this is consistent with the lack of any observable changes in epinephrine levels previously reported for rest in response to altitude or hypoxic exposure alone (3,5,14). These resting data without caffeine ingestion appear consistent with a cosecretion mechanism for ECPs and epinephrine from the adrenal medulla. Conversely, caffeine ingestion of this dosage has been demonstrated to induce significant increases in resting epinephrine levels in the blood

(2,13,16). Thus, it was hypothesized that increases in resting plasma Peptide F ir levels would result with caffeine ingestion. If ECPs are responsive to the same stimuli that results in epinephrine release (6,12,17), our data then suggest the mechanism for these responses may involve non-colinear release of epinephrine and Peptide F. Further direct study is needed examining epinephrine and ECPs plasma release patterns with caffeine ingestion at rest to elucidate these findings.

Without caffeine ingestion, this study demonstrated that significant increases in plasma Peptide F ir levels occurred only with chronic altitude exposure. This may indicate an adaptive timecourse in adrenal medulla release mechanisms during exercise with altitude acclimatization. Previous studies examining plasma Peptide F ir responses to exercise had demonstrated significant increases in response to a 7 min exercise stage utilizing an exercise intensity of 83% of $\dot{V}O_2\text{max}$ (7,8). Subjects utilized in these previous studies had been acclimatized (> 2 yr residence) to moderate altitude and were tested at 2200 m. Thus, the acclimatization process to altitude appears to influence the exercise responses to high intensity submaximal endurance exercise. Still, it has been established that such high intensity exercise (80 to 85% of $\dot{V}O_2\text{max}$) at sea level and moderate altitude (2200 m) elicits a substantial increase in epinephrine concentrations in the plasma (5,6). Thus, the lack of any exercise-induced increases in Peptide F ir at sea level and with acute altitude exposures, again supports the possibility of a non-colinear release mechanism for epinephrine and ECPs in response to exercise. Furthermore, this mechanism may be altered over the course of altitude acclimatization.

Previous studies have demonstrated caffeine to increase resting levels of Beta-endorphin (1,15). Our study demonstrated that caffeine ingestion ($4\text{mg}\cdot\text{KgBW}^{-1}$) significantly increased only exercise and post-exercise plasma Peptide F

ir values at sea level, and exercise values with acute altitude exposure. While not effecting resting levels, the pattern for caffeine's effects on plasma Peptide F ir during and following exercise appears to be different at each altitude exposure. Post-exercise values for plasma Peptide F ir after caffeine ingestion were significantly lower at both acute and chronic altitude exposures compared to sea level. This suggests possible differences in exercise recovery mechanisms after caffeine ingestion. Furthermore, caffeine ingestion significantly decreased the response of Peptide F measured post-exercise at chronic altitude compared to the "no caffeine" response at that timepoint. These results may indicate that caffeine may interact with the acclimatization process and alter the exercise recovery release mechanisms after altitude acclimatization.

In summary, our data suggest that caffeine and altitude interact to produce different exercise and recovery response patterns of plasma Peptide F ir levels. Furthermore, altitude acclimatization may play a significant role in altering the release mechanisms involved with the exercise stress response and recovery. The exact mechanisms remain unknown but strongly implicates further study examining the relationships with catecholamine release, and more specifically epinephrine. The effects of different exercise intensities at sea level on Peptide F ir responses requires further study. Still, it is evident from our study that a complex system for preproenkephalin release and processing is involved consequent caffeine ingestion, altitude exposure and exercise stresss.

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Figure 1. Peptide F ir responses to exercise "without" (open bars) and "with" (shaded bars) caffeine ingestion are presented.

*= $p < 0.05$ with corresponding pre-exercise value.

Δ = $p < 0.05$ with corresponding without caffeine value, and

+= $p < 0.05$ with corresponding sea level value.

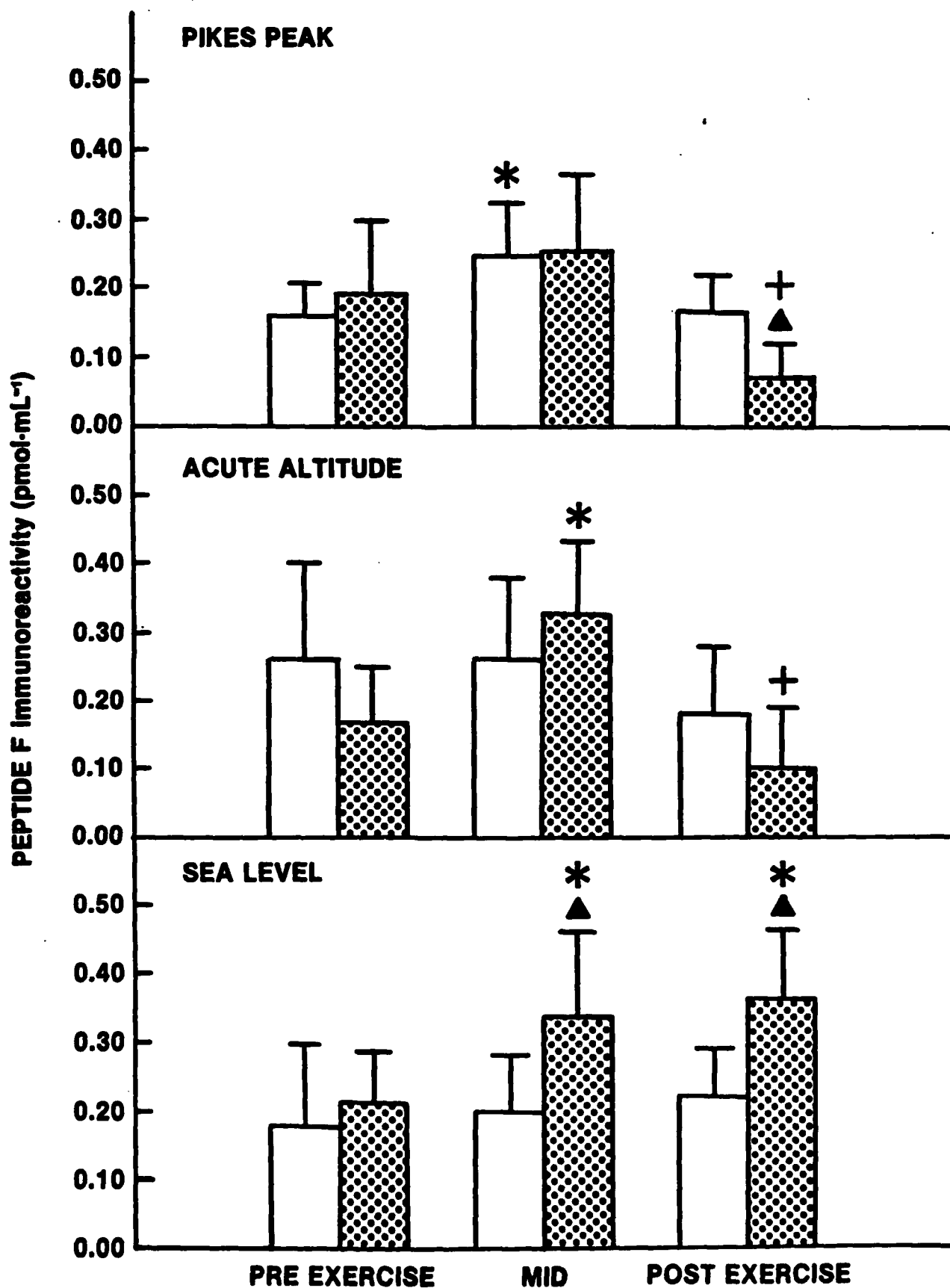


Table 1. Mean ($\pm 1SD$) metabolic and cardiovascular responses to high intensity submaximal exercise at three altitude exposures.

| | SEA LEVEL | | | ACUTE ALTITUDE | | | PIKES PEAK | | |
|---|--------------------|-------------------|-------------------|------------------|-------------------|-------------------|------------------|--------------------|--------------------|
| | PRE- | MID- | POST-EX | PRE- | MID- | POST-EX | PRE- | MID- | POST-EX |
| BLOOD LACTATE | NC 1.26 (0.24) | 8.67* (1.10) | 8.69* (1.93) | 1.66 (0.28) | 6.78* (1.29) | 8.02* (2.31) | 1.70 (0.31) | 4.72*# (0.69) | 5.58*# (1.85) |
| | C 1.48 (0.27) | 8.69* (1.84) | 8.69* (2.27) | 2.08 (0.52) | 7.42* (1.09) | 9.81* (2.33) | 1.40 (0.22) | 4.69*# (1.15) | 4.75* (1.24) |
| HEART RATE (beats min ⁻¹) | NC 66.88 (9.18) | 175.88* (8.85) | 185.0* (7.94) | 77.44 (12.72) | 166.77* (5.11) | 171.75* (6.73) | 88.25 (11.37) | 159.60*# (3.89) | 172.50*# (6.26) |
| | C 71.87 (8.74) | 178.25 (11.92) | 184.55* (8.41) | 73.98 (11.29) | 169.16* (6.64) | 178.28* (6.23) | 86.37 (13.56) | 161.43*# (6.02) | 176.62*# (6.02) |
| OXYGEN UPTAKE (L \cdot min ⁻¹) | NC 0.37 (0.08) | 3.15* (0.44) | 3.19* (0.42) | 0.41 (0.05) | 2.34* (0.40) | 2.68* (0.48) | 0.40 (0.08) | 2.32*# (0.43) | 2.43*# (0.43) |
| | C 0.38 (0.08) | 3.28* (0.52) | 3.23* (0.40) | 0.43 (0.07) | 2.34* (0.38) | 2.60* (0.39) | 0.42 (0.11) | 2.27*# (0.39) | 2.43*# (0.36) |
| % Δ PLASMA VOLUME (Pre-to Post-Ex) | NC | -11.24 (6.10) | | | -6.38# (4.15) | | | +0.68*# (6.71) | |
| | C | -10.96 (6.10) | | | -4.78# (8.29) | | | +1.53*# (10.71) | |

NC = no caffeine, C = caffeine, * = $p < 0.05$ from corresponding within group resting value, # = $p < 0.05$ from corresponding sea level value, + = $p < 0.05$ from corresponding acute altitude value.

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